Modeling the Irradiation Followed by Heat Inactivation of *Salmonella* Inoculated in Liquid Whole Egg

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ABSTRACT: This study presents mathematical models that describe the inactivation of *Salmonella* Enteritidis, *Salmonella* Typhimurium, and *Salmonella* Senftenberg suspended in liquid whole egg (LWE) by irradiation followed by heat treatments (IR-H treatments). These models also enable prediction of cell injury in *Salmonella* after exposure to IR-H. *Salmonella* viability decreased exponentially (primary model) with heat treating time for all the radiation doses (0, 0.1, 0.3, 0.5, 1.0, and 1.5 kGy) and temperatures investigated (55, 57, and 60 °C). Two secondary models that related the D_T values (time required to eliminate 90% of viable cells at a given temperature) with radiation dose, heating temperature, and recovery medium after treatments were also developed. The developed final equations enabled to establish the *process criterion* (combinations of irradiation doses, temperature, and heat treatment times) required to achieve a given reduction (*performance criterion*) in *Salmonella* spp. suspended in LWE or the cell damage caused by the treatments. Process criteria to obtain the established performance criteria (a 5-$\log_{10}$ reduction) on any of the investigated *Salmonella* serovars were determined to be, 57.7 °C/3.5 min following 1.5 kGy when treated cells were recovered in tryptic soy agar and 59.3 °C/3.5 min following 0.5 kGy when cells were recovered in tryptic soy agar amended with 3% NaCl. Based on our results, current industrial LWE heat treatments (60 °C/3.5 min) would inactivate 3 $\log_{10}$ cycles of the *Salmonella* population. The results of this study can be applied to engineering design and for the evaluation and optimization of the IR-H process as a new technique to obtain *Salmonella*-free LWE.

Keywords: heat, irradiation, liquid whole egg, microbial inactivation, *Salmonella*

Introduction

Based on the Intl. Commission on Microbiological Specifications for Foods (ICMSF) recommendations, the design of effective preservation processes that assure the safety and stability of foods requires, among other things, the establishment of the treatment conditions (*process criterion*) required to obtain a predetermined number of $\log_{10}$ reductions of the target pathogenic microorganism (*performance criterion*) (van Schothorst 1998; Stewart and others 2002). For liquid whole egg (LWE), heat pasteurization treatment conditions are well defined by the Food and Drug Administration (FDA) (minimum 60 °C for 3.5 min—Code of Federal Regulation [CFR 2000, Sec. 590.570]). However, the FSIS (Food Safety Inspection Service) has indicated that these current minimum time and temperature requirements for pasteurization of egg products are not adequate to ensure that no *Salmonella* will survive pasteurization (FSIS 1998). Therefore, more intensive heat treatments would be necessary to assure the safety and security of LWE, which would unfortunately reduce its quality since some soluble proteins begin to precipitate at temperatures as low as 57 °C (Hamid-Samimi and others 1984; Herald and Smith 1989). Alternative nonthermal processes have been proposed to substitute heat pasteurization of LWE (Jeantet and others 1999; Lee and others 2003; Mañas and others 2003). Ionizing radiation is a nonthermal process that can be potentially used to inactivate *Salmonella* spp. in liquid whole egg (Proctor and others 1953; Schaffner and others 1989; Serrano and others 1997). However, using irradiation alone also can be prohibitive since the doses proposed (2 to 3 kGy) to achieve a 5-$\log_{10}$ reduction in the population of *Salmonella* spp. also can adversely affect the quality of LWE, as undesirable effects have been reported at radiation doses of 1.5 kGy (Serrano and others 1997).

Combining heat and irradiation can potentially alleviate the severity of treatment for LWE, thus improving the product quality. Recent results obtained in our lab indicated that the combination of irradiation followed by heat would be a promising technology to obtain *Salmonella*-free LWE by applying irradiation doses lower than 1.5 kGy and maximum temperatures of 60 °C (Alvarez and others 2006). However, designing an effective irradiation followed by heat process (IR-H process) that assures the safety and stability of LWE requires establishing the corresponding *performance* and *process criteria*.

In order to establish the treatment conditions of the IR-H process, it is necessary to determine the kinetics of microbial inactivation and to describe them mathematically. From this description, predictive mathematical models can be developed in the course of establishing the *process criterion*. These models and criteria are used for the design and construction of the processing equipment and for the development of Hazard Analysis Critical Control Point (HACCP) plans (Ross and McMeekin 1994; Linton and others 1995; McMeekin and others 2002). To develop a predictive model it is necessary to describe, in a 1st step, the microbial evolution as function of time (primary model). Then, parameters appearing in primary models

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are expressed as functions of process parameters and environmental conditions (secondary model). Finally, primary and secondary models are combined to predict the microbial evolution as functions of time, process parameters, and environmental conditions (final equation) (McMeekin and Ross 2002).

One of the environmental conditions that has more influence on the microbial inactivation kinetics parameters is the condition of microbial recovery after treatments. Depending on the recovery conditions (that is, temperature of incubation, recovery in selective media, and so on) the number of surviving cells to a treatment will vary (Borsa and others 1995; Chawla and others 1996). Judicious use of appropriate recovery media enables the detection and quantization of cell injury. The development of mathematical models that allows quantization of cell damage after treatments is essential to extend the use of the hurdle technology in the food industry (Raso and Barbosa-Cánovas 2003).

The objective of this investigation was to develop a mathematical model that describes the inactivation of two of the *Salmonella* serovars most frequently reported as causing illness, serotype Enteritidis and Typhimurium (Anonymous 2002), and that of one of the most heat- and irradiation-resistant *Salmonella* serovars, *Salmonella* Senftenberg (Mañas and others 2003; Álvarez and others 2005), suspended in LWE following irradiation and heat treatments. The developed mathematical model will enable LWE producers to maximize the lethality of the process while minimizing the intensity of the treatments to achieve a predetermined *performance criterion*, and to determine the *process criterion* that induces damage of *Salmonella* cells after an IR-H process in LWE.

Material and Methods

Extra-large grade A eggs were purchased from a local supermarket. Egg shells were thoroughly washed with 70% ethanol and allowed to air dry. Sanitized eggs were aseptically broken and transferred into a sterile stomacher bag (Tekmar Co., Cincinnati, Ohio, U.S.A.) and homogenized for 2 min in a stomacher laboratory blender 400 (Tekmar Co., Cincinnati, Ohio, U.S.A.). The obtained liquid whole egg was maintained at 2 to 4 °C until ready for use.

Microorganism

Three serovars of *Salmonella* were used for irradiation and heat treatments: *Salmonella* Enteritidis 13076; *Salmonella* Senftenberg 8400; and *Salmonella* Typhimurium 14028 (American Type Culture Collection, Manassas, Va., U.S.A.). Stock cultures were maintained in tryptic soy agar (TSA) (Difco, Sparks, Md., U.S.A.) at 2 to 4 °C and transferred monthly.

Bacterial cultures and inoculation

Each *Salmonella* serovar was cultured independently in 50 mL of tryptic soy broth (TSB) (Difco) in 250-mL Erlenmeyer culture flasks at 37 °C (150 rpm) for 18 h. After this incubation time, cells were at their stationary phase of growth. Before treatments, microorganisms were centrifuged at 3500 *g* for 10 min at 4 °C. The TSB was then removed and the pellet was resuspended in LWE. The final cell concentration of the LWE was 10^9 colony forming units (CFU) per milliliter for each *Salmonella* serovar.

Treatments

Irradiation treatments. Borosilicate glass test tubes (16 × 125 mm) containing 2 mL of LWE with 10^9 CFU/mL *Salmonella* were placed vertically in the sample chamber of a Lockheed Georgia Co. self-contained ^{137}Cs irradiator (Marietta, Ga., U.S.A.), with a dose rate of 0.095 kGy/min. The samples were treated at 0, 0.1, 0.3, 0.5, 1.0, and 1.5 kGy. The absorbed dose was verified using 5-mm alanine pellet dosimeters (Brucker, Inc. Billarica, Mass., U.S.A.), and then was measured using a Brucker EMS 104 EPR Analyzer. The temperature during irradiation was maintained at 4 ± 1.0 °C. Temperature control was maintained by thermocouple-controlled injection of gas-phase liquid nitrogen into the sample chamber.

Heat treatments. Heat treatments were carried out by inoculating 0.2 mL of LWE with 10^9 CFU/mL of the corresponding bacterial suspension in test tubes (16 × 125 mm borosilicate glass) containing 2 mL of liquid whole egg, that had been previously stabilized in a water bath (General Purpose Aquabath™ model 18007, Lab Line, Melrose Park, Ill., U.S.A.) at the desired temperature (55, 57, and 60 ± 0.2 °C). Actual temperature was controlled using a thermocouple wire introduced in a 2-mL test tube that contained LWE, immersed in the water bath. At preset time intervals, samples were taken for the heat resistance determinations and immediately submerged in an ice–water bath in order to rapidly cool down.

Irradiation followed by heat treatments (IR-H treatments). Test tubes containing 2 mL of LWE with 10^9 CFU/mL of the bacterial suspension were treated at 0, 0.1, 0.3, 0.5, 1.0, and 1.5 kGy. The samples were held at 4 °C before, during, and after irradiation. Irradiated LWE (0.2 mL) inoculated with bacteria was added in test tubes containing 2-mL LWE, previously stabilized in a water bath at the desired temperature (55, 57, and 60 °C). The following steps were similar to those described for the heat treatments. The time between irradiation and heat treatments was 10 to 60 min. This period had no effect on the number of survivors after irradiation treatments or the heat resistance of *Salmonella* serovars (data not shown).

Sampling

Two aliquots (0.1 mL) of LWE treated by irradiation, heat, or irradiation followed by heat, and nontreated samples were removed and serially diluted with Butterfield's phosphate buffer of pH 7. Pour plating with TSA was carried out to determine the surviving bacterial population. Two pour plates per dilution were incubated for 48 h at 37 °C. When injury was investigated, TSA with 3% (wt/vol) sodium chloride (NaCl) added (TSA-SC) was used as the recovery medium, which prevented the recovery of membrane damaged cells after the applied treatments. This medium did not affect the viability of nontreated cells (data not shown). The TSA-SC plates were incubated for 48 h at 37 °C. After incubation, the colonies were counted with an automatic plate laser counter (Exotech Inc., Gaithersburg, Md., U.S.A.). Differences on counts between cells recovered in TSA and TSA-SC enabled the determination of the number of damaged cells after treatments. All experiments were replicated 3 times.

Irradiation and heat resistance parameters

Plate counts of the irradiation-treated samples were divided by the control plate counts to give a survival fraction (S_{IR}). The $\log_{10}$ of the survival fraction was then used for determination of $D\gamma$ values (radiation dose required to eliminate 90% of the viable cells). $D\gamma$ values were also obtained when treated cells were recovered in TSA-SC. The lethality of heat treatments was measured by their decimal reduction time value (D_T value), which is defined as minutes of treatment at a given temperature to eliminate 90% of the viable cells. D_T values were calculated from the slope of the regression line of the straight portion of the survival curve obtained at every treatment temperature by plotting $\log_{10}$ of the survival fraction compared with treatment time. $D\gamma$, D_T values, their corresponding 95% confidence limits, and 1-way analysis of variance (ANOVA) were determined with GraphPad PRISM® (GraphPad Software Inc., San Diego, Calif., U.S.A.).

Model development

Microbial inactivation after irradiation treatments was determined by the following primary model (Eq. 1):

$$\mathrm{Log}_{10} S_{IR} = -\frac{IR}{D_\gamma} \quad (1)$$

where S_{IR} is the survival fraction and IR the irradiation dose (kGy).

After an irradiation treatment, the lethality of heat treatments at a determined temperature (T) was also described by a similar primary model (Eq. 2):

$$\mathrm{Log}_{10} S_H = -\frac{x}{D_T} \quad (2)$$

where S_H is the survival fraction and x is the treatment time (min).

The relationships between the D_T values and the irradiation dose, temperature, and recovery medium for each *Salmonella* serovar were modeled by 2 different secondary models. The secondary model I was based on the traditional 1st-order inactivation kinetics of heat treatments, in which a linear relationship is observed between the $\log_{10}$ of the D_T values and the temperature of the heat treatment (decimal reduction time curve—DRTC). A total of 12 DRTC curves were obtained for each *Salmonella* serovar, one for each irradiation dose (6) and recovery condition (2). Each DRTC was defined by its z-value (reciprocal of the slope or °C increase necessary to reduce D_T value a $\log_{10}$ cycle) and the Y intercept, which is a function of the irradiation dose and the recovery conditions (Eq. 3).

$$\mathrm{Log}_{10} D_T = -\frac{1}{z} T + Y \quad (3)$$

where T is the temperature of the heat treatment (°C).

The effect of the irradiation dose and the recovery media on the Y intercept was determined by a quadratic polynomial equation generated with Statgraphics Plus 5.1 (Statistical Graphics Corp., Va., U.S.A.). Backward stepwise regression was used to eliminate the parameters that were not significant to the model. This regression procedure begins with all candidate variables in the model and then systematically removes variables that are not statistically significant ($P > 0.01$) (Gómez and others 2005). The objective is to determine a model that induces only statistical significant terms since including insignificant terms increases the deviation of the predictions (Juneja and others 2003).

The significance of the differences between the z-values at each irradiation dose and the Y intercepts at the different irradiation doses for each serovar was determined by ANOVA (GraphPad PRISM®).

The secondary model II is a quadratic polynomial model also generated with Statgraphics Plus 5.1. This model includes as parameters the temperature, the irradiation dose, and the percentage of NaCl in the recovery medium. The coefficients for the parameters were calculated with a multiparametric linear regression using the statistic software Statgraphics Plus 5.1.

To compare the proposed secondary models, the correlation coefficient (R^2), the root mean square error (RMSE), and the accuracy factor (Ross 1996; Baranyi and others 1999) were determined. The accuracy factor (A_f) indicates how many predictions differ from the observed data. An accuracy factor equal to 1 indicates that predictions of the model have not a mean discrepancy compared with the obtained data (Baranyi and Pin 2001). The A_f values were calculated using the following equation:

$$A_f = 10^{\sqrt{\frac{1}{n}\sum_{i=1}^{n}\left[\mathrm{Log}_{10}\left(\frac{predicted_{(i)}}{observed_{(i)}}\right)\right]^2}}$$

were n is the number of observations. The A_f value can be expressed in percentages of discrepancy or error (%D) (Baranyi and Pin 2001) between observed and predicted data with the following equation:

$$\%\mathrm{D} = (A_f - 1)100$$

F-tests were also done to compare the fit of the secondary models, using GraphPad PRISM®, since F-test accounts for the difference in the number of parameters contained in the model besides its best fit.

The total microbial inactivation for each *Salmonella* serovar after IR-H treatments (S_{IR-H}) was calculated with the final equation by adding the lethal effect of irradiation treatments (S_{IR}) and the heat treatments after irradiation (S_H) (Eq. 4):

$$\mathrm{Log}_{10} S_{IR-H} = \mathrm{Log}_{10} S_{IR} + \mathrm{Log}_{10} S_H \quad (4)$$

Results and Discussion

Inactivation of *Salmonella* by irradiation followed by heat

The survival curves after irradiation treatments, corresponding to the inactivation of the 3 *Salmonella* serovars suspended in LWE, are shown in Figure 1. $D\gamma$ values of 0.49 ± 0.01, 0.65 ± 0.06, and 0.44 ± 0.04 kGy were obtained for *Salmonella* Enteritidis, *Salmonella* Senftenberg, and *Salmonella* Typhimurium, respectively. Significant differences ($P < 0.05$) were observed among $D\gamma$ values of *Salmonella* Enteritidis and Typhimurium and that of *Salmonella* Senftenberg. Similar *Salmonella* $D\gamma$ values were reported in the literature on LWE and other food products (Proctor and others 1953; Monk and others 1994; Sherry and others 2004). When cells were recovered in TSA-SC, $D\gamma$ values of 0.51 ± 0.07, 0.64 ± 0.02, and 0.42 ± 0.04 kGy were obtained for *Salmonella* Enteritidis, *Salmonella* Senftenberg, and

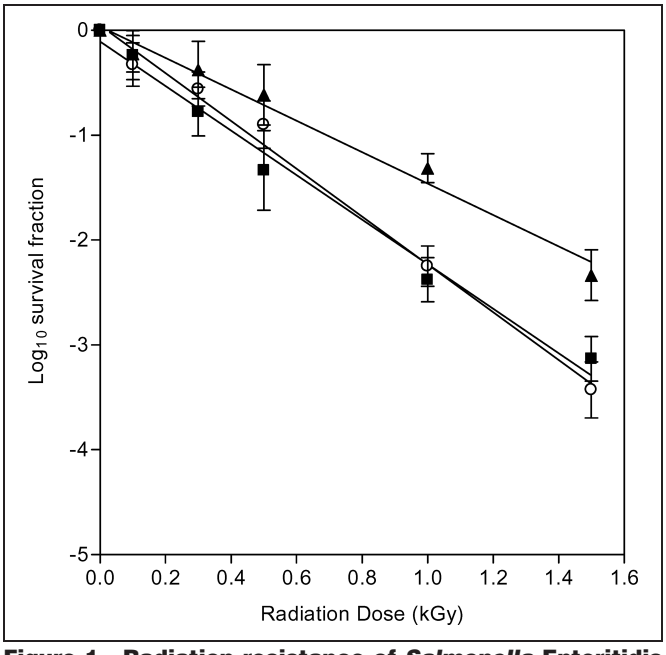

Figure 1—Radiation resistance of *Salmonella* Enteritidis (■), *Salmonella* Senftenberg (▲), and *Salmonella* Typhimurium (○) suspended in LWE. Linear regressions are shown as solid lines. 95% confidence intervals are indicated with error bars.

Table 1 — D_T values (in minutes) from heat treatments following irradiation for *Salmonella* serovars treated in LWE and recovered in TSA and TSA-SC

		Salmonella Enteritidis		*Salmonella* Senftenberg		*Salmonella* Typhimurium	
Dose (kGy)	Temperature (°C)	D_T–TSA[a]	D_T–TSA-SC[b]	D_T–TSA[a]	D_T–TSA-SC[b]	D_T–TSA[a]	D_T–TSA-SC[b]
0	55	7.04 ± 0.03	6.62 ± 0.27	11.31 ± 0.24	7.01 ± 0.68	6.63 ± 0.57	6.53 ± 0.52
	57	3.39 ± 0.11	3.32 ± 0.31	4.33 ± 0.14	4.20 ± 0.14	3.16 ± 0.17	2.86 ± 0.12
	60	0.63 ± 0.03	0.58 ± 0.06	1.14 ± 0.07	1.09 ± 0.03	0.58 ± 0.07	0.58 ± 0.08
0.1	55	3.50 ± 0.09	3.10 ± 0.05	3.18 ± 0.15	2.26 ± 0.08	3.37 ± 0.23	2.97 ± 0.84
	57	2.07 ± 0.39	1.60 ± 0.20	1.75 ± 0.23	1.55 ± 0.05	2.31 ± 0.22	2.26 ± 0.28
	60	0.87 ± 0.18	0.56 ± 0.06	0.61 ± 0.01	0.60 ± 0.01	1.09 ± 0.08	1.04 ± 0.25
0.3	55	3.83 ± 0.27	3.54 ± 0.39	3.50 ± 0.45	1.97 ± 0.54	4.41 ± 0.75	4.01 ± 0.60
	57	1.83 ± 0.29	1.67 ± 0.16	1.60 ± 0.25	1.69 ± 0.16	2.26 ± 0.15	1.87 ± 0.04
	60	0.99 ± 0.05	0.83 ± 0.02	0.55 ± 0.01	0.49 ± 0.05	1.20 ± 0.08	1.02 ± 0.08
0.5	55	5.07 ± 0.46	4.23 ± 0.23	3.39 ± 0.80	2.81 ± 0.27	3.89 ± 0.43	4.08 ± 0.10
	57	2.16 ± 0.09	2.27 ± 0.25	1.27 ± 0.29	1.23 ± 0.13	2.53 ± 0.24	2.02 ± 0.12
	60	1.21 ± 0.18	0.82 ± 0.12	0.64 ± 0.12	0.54 ± 0.02	1.09 ± 0.14	0.74 ± 0.04
1.0	55	5.44 ± 1.04	4.69 ± 0.55	2.46 ± 0.12	2.23 ± 0.31	3.80 ± 0.13	3.23 ± 0.61
	57	2.12 ± 0.54	2.13 ± 0.41	1.65 ± 0.10	1.33 ± 0.26	2.14 ± 0.11	2.05 ± 0.08
	60	1.10 ± 0.08	0.90 ± 0.07	0.63 ± 0.04	0.59 ± 0.05	0.76 ± 0.15	0.51 ± 0.11
1.5	55	6.54 ± 0.27	5.77 ± 0.19	2.64 ± 0.55	1.97 ± 0.15	2.57 ± 0.59	1.73 ± 0.59
	57	2.60 ± 0.21	2.05 ± 0.40	1.58 ± 0.03	1.58 ± 0.05	2.16 ± 0.13	1.74 ± 0.08
	60	1.20 ± 0.17	1.12 ± 0.09	0.53 ± 0.01	0.58 ± 0.04	0.65 ± 0.14	0.58 ± 0.12

[a] D_T values obtained from survival curves in which cells were recovered in TSA.
[b] D_T values obtained from survival curves in which cells were recovered in TSA added with 3% NaCl.
95% confidence limits are indicated with the ± values.

Salmonella Typhimurium, respectively. No significant differences ($P > 0.05$) were observed among $D\gamma$ values determined in TSA or TSA-SC for each serovar, indicating that no membrane damage was detected after irradiation treatments. Therefore, $D\gamma$ mean values of 0.50, 0.64, and 0.43 kGy for *Salmonella* Enteritidis, *Salmonella* Senftenberg, and *Salmonella* Typhimurium were used, respectively, to determine the *Salmonella* inactivation after irradiation doses in LWE.

Survival curves corresponding to heat treatments at 55, 57, and 60 °C of *Salmonella* serovars Enteritidis, Senftenberg, and Typhimurium previously treated by ionizing radiation (0 to 1.5 kGy) were linear for all the combinations investigated (data not shown). The influence of irradiation on the thermal resistance of the *Salmonella* serovars was determined. Table 1 summarizes D_T values obtained after different IR-H treatments for each *Salmonella* serovar when recovered in TSA and TSA-SC. The 95% confidence limits of the corresponding D_T values have also been included to illustrate the precision of the results. When only heat was applied, *Salmonella* Senftenberg showed the highest D_t values among the investigated serovars at all temperatures studied. The heat resistance observed was within the range of most published data for these serovars (Doyle and Mazzotta 2000). D_T values decreased linearly with temperature for each irradiation dose. $D_{60\,°C}$ values of *Salmonella* Enteritidis and *Salmonella* Typhimurium obtained after irradiation treatments were higher than those obtained after heating at 60 °C without preirradiation. This would indicate that irradiation followed by heat treatments at 60 °C induced a cross-protection phenomenon on these *Salmonella* serovars as was described by Álvarez and others (2006). The existence of a cross-protection phenomenon could pose a major problem to the LWE industry since these 2 serovars are the most common *Salmonella* isolates in foodborne outbreaks (Anonymous 2002). However, their high sensitivity to irradiation and the synergistic effect of the combination treatments at the lowest heat temperatures investigated would permit the reduction of heat treatment times to achieve a predetermined level of pathogen inactivation of these serovars in LWE when applying IR-H processes. From a practical point of view, the development of mathematical models that enable prediction of *Salmonella* inactivation in LWE after IR-H treatments, or of the temperatures over which the cross-protection phenomenon occurs, would be of great interest to establish the process criterion to achieve a determined performance criterion for *Salmonella* in LWE.

Model development

Primary model. Microbial inactivation after irradiation doses was determined with Eq. 1. In this equation, $D\gamma$ was specific for each *Salmonella* serovar, and independent of the recovery medium. Therefore, $D\gamma$ mean values of 0.50, 0.64, and 0.43 kGy for *Salmonella* Enteritidis, *Salmonella* Senftenberg, and *Salmonella* Typhimurium were considered, respectively.

Equation 2 describes the *Salmonella* inactivation by heat treatments. D_T values varied with the *Salmonella* serovar, irradiation dose, temperature, and recovery medium (Table 1). A total of 36 D_T values were obtained for each serovar (2 recovery media, 3 temperatures, and 6 irradiation doses). Therefore, secondary models that correlated the thermal resistance with the other parameters could be generated.

Secondary model. Two secondary models were developed to describe the effect of irradiation dose, temperature, and recovery medium on D_T values. Equation 3 (secondary model I) expressed the D_T values as an exponential function of the temperature, irradiation dose, and NaCl in the recovery medium. For each *Salmonella* serovar, the thermo-dependence of the D_T values was characterized by a z-value for each irradiation dose and recovery medium and the corresponding Y intercept of the DRTC (Table 2). When only heat was applied, the z-value was similar to that reported for most vegetative cells investigated (Lovett and others 1982; Schuman and Sheldon 1997; Pagán and others 1998; Juneja 2003). Significant differences ($P < 0.05$) were observed between z-values of heat treatments and those obtained from heat treatments following irradiation. This would indicate that mechanisms involved in *Salmonella* inactivation after irradiation and heat treatments would be different from those of heat treatments based on the cross-protection phenomenon. On the other hand, since the slopes of the DRTC corresponding to irradiation followed by heat treatments were not significantly different ($P > 0.05$), it was possible to calculate 1 slope for all the data, and therefore the z-values were independent of the irradiation dose and the recovery medium for each *Salmonella* serovar. A z_{MEAN} value for all the DRTC and characteristic of each serovar was determined (Table 2). However, the differences between Y intercepts

Table 2—*z*-Values (°C) and *Y* intercepts from the fitting of the secondary model I to the D_T values obtained after heat treatments following irradiation of the 3 *Salmonella* serovars investigated, recovered in TSA and TSA-SC

		0 kGy		0.1 kGy		0.3 kGy		0.5 kGy		1.0 kGy		1.5 kGy	
		z^a	Y^b	z^a	Y^b	z^a	Y^b	z^a	Y^b	z^a	Y^b	z^a	Y^b
Salmonella Enteritidis	TSA	4.7 ± 0.4	12.6 ± 1.2	8.2 ± 0.2	7.2 ± 0.2	8.7 ± 1.8	6.9 ± 1.1	8.2 ± 1.5	7.3 ± 1.6	7.4 ± 1.4	8.1 ± 1.7	6.9 ± 1.4	8.7 ± 1.4
	TSA-SC	4.6 ± 0.9	12.8 ± 2.1	6.7 ± 0.3	8.7 ± 0.1	8.1 ± 1.3	7.4 ± 1.0	7.0 ± 0.2	8.5 ± 0.2	7.0 ± 0.7	8.5 ± 0.7	7.3 ± 1.9	8.3 ± 2.1
		$z_{MEAN} = 7.5\,^{\circ}C$											
		$Y = 7.9 + 0.1 \cdot IR - 0.07 \cdot SC + 0.2 \cdot IR \cdot SC(1 - IR)$											
Salmonella Senftenberg	TSA	5.0 ± 0.2	12.0 ± 0.3	6.9 ± 0.3	8.4 ± 0.4	6.2 ± 0.2	9.4 ± 0.2	7.1 ± 2.0	8.2 ± 1.8	8.3 ± 1.2	7.1 ± 0.9	7.1 ± 0.7	8.2 ± 0.7
	TSA-SC	6.1 ± 1.0	9.9 ± 1.3	8.5 ± 1.2	6.8 ± 0.8	7.9 ± 1.9	7.3 ± 2.3	7.6 ± 0.6	7.7 ± 0.6	8.7 ± 0.1	6.7 ± 0.1	9.2 ± 2.0	6.3 ± 1.5
		$z_{MEAN} = 7.7\,^{\circ}C$											
		$Y = 7.7 - 0.04 \cdot IR - 0.03 \cdot SC + 0.01 \cdot IR^2 \cdot SC$											
Salmonella Typhimurium	TSA	4.8 ± 0.2	12.3 ± 0.8	10.1 ± 0.8	6.0 ± 0.4	9.8 ± 2.1	6.3 ± 1.1	9.0 ± 0.6	6.7 ± 0.4	7.1 ± 0.4	8.3 ± 0.4	8.1 ± 3.5	7.2 ± 2.1
	TSA-SC	4.7 ± 0.3	12.5 ± 0.8	10.8 ± 1.5	5.6 ± 0.8	9.3 ± 2.8	6.5 ± 1.4	6.8 ± 1.1	8.7 ± 0.1	6.1 ± 1.3	9.6 ± 1.6	10.0 ± 3.0	5.8 ± 2.5
		$z_{MEAN} = 8.4\,^{\circ}C$											
		$Y = 7.1 - 0.03 \cdot IR \cdot SC - 0.06 \cdot IR^2$											

[a] *z*-value.
[b] *Y* intercept from DRTC.

were significant ($P < 0.05$) and depended on the irradiation dose and the recovery medium. Quadratic polynomial equations shown in Table 2 for each *Salmonella* serovar described the relationship between the *Y* intercepts of DRTC with the irradiation dose and the recovery media. A final secondary model I for each *Salmonella* serovar was developed by including z_{MEAN} and *Y* intercept equation in Eq. 3:

Salmonella Enteritidis:

$$\mathrm{Log}_{10} D_T = -\frac{T}{7.5} + [7.9 + 0.1 \cdot IR - 0.07 \cdot SC + 0.2 \cdot IR \cdot SC\,(1 - IR)]$$

Salmonella Senftenberg:

$$\mathrm{Log}_{10} D_T = -\frac{T}{7.7} + (7.7 - 0.04 \cdot IR - 0.03 \cdot SC + 0.01 \cdot IR^2 \cdot SC)$$

Salmonella Typhimurium:

$$\mathrm{Log}_{10} D_T = -\frac{T}{8.4} + (7.1 - 0.03 \cdot IR \cdot SC - 0.06 \cdot IR^2)$$

where *T* is the temperature of the heat treatments (from 55 to 60 °C), *IR* is the irradiation dose (from 0.1 to 1.5 kGy), and *SC* is the presence of NaCl (3%) in the recovery medium.

Data from Table 1 were used to generate quadratic polynomial models (secondary model II) that enabled us to describe the D_T values as a function of the temperature (from 55 to 60 °C), irradiation dose (from 0.1 to 1.5 kGy), and recovery medium (TSA or TSA + 3% NaCl). For each *Salmonella* serovar, the following secondary models II were obtained:

Salmonella Enteritidis:

$$D_T = 280 + 528 \cdot IR - 0.1 \cdot SC - 9.2 \cdot T + 0.1 \cdot T^2 - 17.9 \cdot IR \cdot T + 0.2 \cdot IR \cdot T^2$$

Salmonella Senftenberg:

$$D_T = 299 - 4.4 \cdot IR - 82 \cdot SC - 9.8 \cdot T + 0.1 \cdot T^2 + 0.1 \cdot IR \cdot T + SC \cdot T \cdot (2.8 - 0.01 \cdot SC \cdot T)$$

Salmonella Typhimurium:

$$D_T = 24 + 1135 \cdot IR - 0.1 \cdot SC - 0.4 \cdot T - 877 \cdot IR^2 + IR \cdot T \cdot (-39 + 0.3 \cdot T + 30 \cdot IR - 0.3 \cdot IR \cdot T)$$

where *T* is the temperature of the heat treatments (°C), *IR* is the irradiation dose (kGy), and *SC* is the presence of NaCl (3%) in the recovery medium (%).

In order to choose the best model, both criteria simplicity of the model and goodness of the fit were considered (Ross and others 1999). According to the principle of parsimony that states that models should contain as few parameters as possible, the secondary model I would be preferable because it was built with fewer parameters. However, the goodness of the fit could represent a more valuable criterion since the simplification of modeling with computers enables use of larger models with several parameters. Table 3 shows the R^2, the RMSE, and the A_f of the 2 fitted secondary models. Based on these coefficients, it can be concluded that both models fit the relationship between the D_T and the temperature, irradiation dose, and the recovery medium reasonably well. However, it appears that the secondary model II is most suitable because it has the highest R^2 and the lowest RMSE. Predictions of model I have a mean discrepancy up to 22% (that is, for *Salmonella* Enteritidis $A_f = 1.224$) when compared with the observed data by only a maximum of 11% with model II. Also *F*-tests done comparing the fits of both models indicated that model II fits best.

Table 3—R^2, RMSE, and A_f factors of the 2 fitted secondary models used to describe the effect of the temperature, irradiation dose, and recovery medium on the D_T values for each *Salmonella* serovar

	Secondary model I			Secondary model II		
	R^{2a}	RMSE[b]	A_f[c]	R^{2a}	RMSE[b]	A_f[c]
Salmonella Enteritidis	0.966	0.377	1.224	0.992	0.206	1.097
Salmonella Senftenberg	0.946	0.218	1.122	0.966	0.181	1.110
Salmonella Typhimurium	0.955	0.336	1.174	0.986	0.191	1.107

[a]Correlation coefficient.
[b]Root mean square error.
[c]Accuracy factor.

To determine the goodness of the fit of the developed secondary model II, the number of $\log_{10}$ cycles of inactivation obtained after the different times, temperatures, and irradiation doses in both recovery media TSA and TSA-SC and those estimated with Eq. 2, in which the secondary model II was included, were utilized (Figure 2). The correlation coefficients of 0.97, 0.95, and 0.97 obtained between observed and estimated data for *Salmonella* Enteritidis, *Salmonella* Senftenberg, and *Salmonella* Typhimurium, respectively, indicated that the predictions obtained with Eq. 2 were a good fit of the observed data.

Final equation. To estimate the survival fraction of any of the 3 *Salmonella* serovars investigated after IR-H processes in LWE, the following final equations were developed by introducing Eq. 1 and 2, with the secondary model II included, in Eq. 4.

Salmonella Enteritidis:

$$\mathrm{Log}_{10} S_{IR-H} = -\frac{IR}{0.50} - x/(280 + 528 \cdot IR - 0.1 \cdot SC - 9.2 \cdot T + 0.1 \cdot T^2 - 17.9 \cdot IR \cdot T + 0.2 \cdot IR \cdot T^2)$$

Salmonella Senftenberg:

$$\mathrm{Log}_{10} S_{IR-H} = -\frac{IR}{0.64} - x/[299 - 4.4 \cdot IR - 82 \cdot SC - 9.8 \cdot T + 0.1 \cdot T^2 + 0.1 \cdot IR \cdot T + SC \cdot T \cdot (2.8 - 0.01 \cdot SC \cdot T)]$$

Salmonella Typhimurium:

$$\mathrm{Log}_{10} S_{IR-H} = -\frac{IR}{0.43} - x/[24 + 1135 \cdot IR - 0.1 \cdot SC - 0.4 \cdot T - 877 \cdot IR^2 + IR \cdot T \cdot (-39 + 0.3 \cdot T + 30 \cdot IR - 0.3 \cdot IR \cdot T)]$$

where S_{IR-H} is the survival fraction after an IR-H process; IR is the applied irradiation dose (kGy); T is the temperature of the heat treatment (°C); x is the treatment time of the heat treatment (min); and SC is the percentage NaCl in the recovery medium (0% or 3%).

The developed final equations enabled us to establish the *process criterion* (irradiation dose followed by heat treatments at a set temperature and time) to achieve a determined *performance criterion* for any of the 3 *Salmonella* serovars studied, and also the *process criterion* that induced injury on *Salmonella* cells after IR-H processes in LWE. Figure 3a and 3b show the combinations of temperature and time of the heat treatments after different irradiation doses to achieve a 5-$\log_{10}$ reduction of the population of any of the investigated *Salmonella* serovars suspended in LWE and

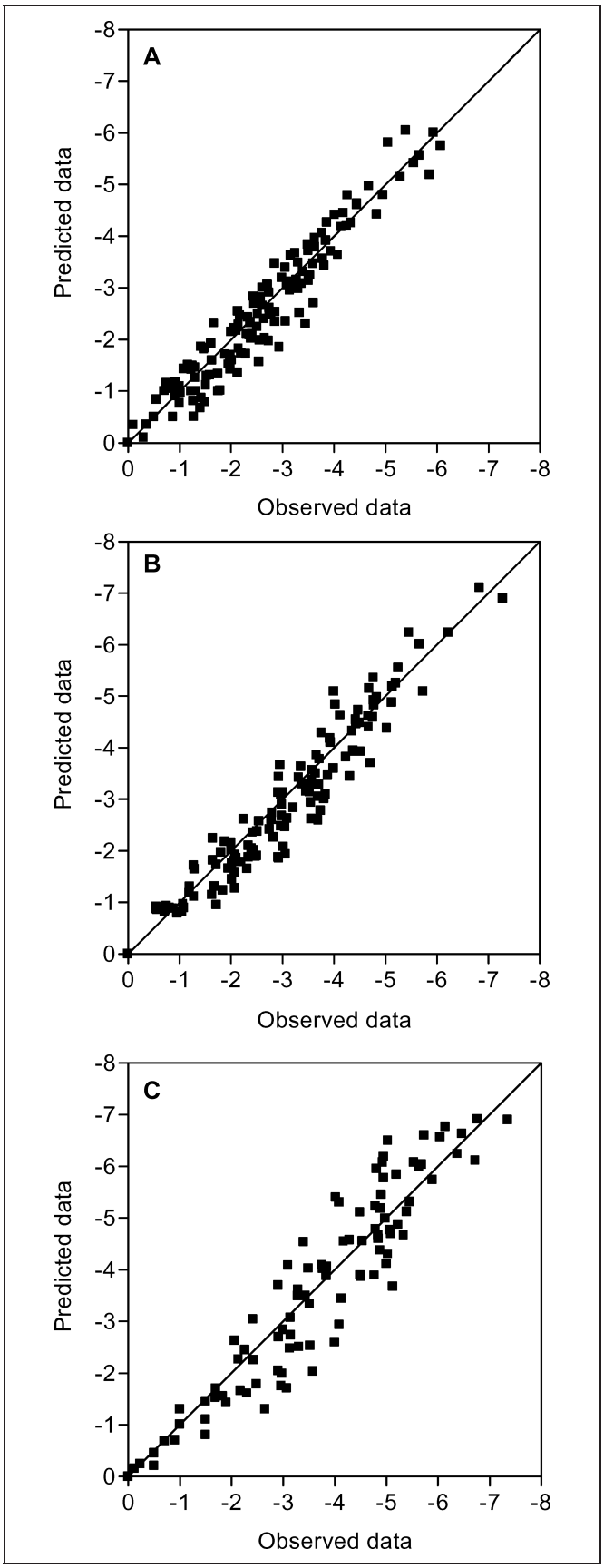

Figure 2—Correlation between observed and estimated $\log_{10}$ cycles of inactivation of (A) *Salmonella* Enteritidis, (B) *Salmonella* Senftenberg, and (C) *Salmonella* Typhimurium estimated with the corresponding developed final equation for each *Salmonella* serovar

recovered in nonselective (TSA) and selective media (TSA-SC), respectively. The comparison between both figures enabled us to determine the cell injured originated by the corresponding IR-H treatments. The thermal death time (TDT) curves for a 5-$\log_{10}$ reduction of the population of the 3 *Salmonella* serovars by heat treatments have been included in both figures. A 3.5-min line corresponding to the industrial heat treatment time used in the United States to pasteurized LWE at 60 °C was also included for comparisons. The combined IR-H process was more effective with respect to the corresponding heat treatment at any temperature and irradiation dose applied to inactivate *Salmonella* (Figure 3a). Based on our results, industrial heat treatments applied in the United States to pasteurized LWE (60 °C/3.5 min) achieved a 5-$\log_{10}$ drop in of the population of *Salmonella* Enteritidis and Typhimurium but only 3 of *Salmonella* Senftenberg, indicating that this treatment would not offer a *Salmonella*-free LWE. However, treatments such as 3.5 min at 58.8 °C following 1.0 kGy or 57.7 °C following 1.5 kGy would enable to inactivate 5 $\log_{10}$ cycles of the population of any of the *Salmonella* serovars investigated. The existence of cell injury after IR-H treatments could reduce the *process criterion* to inactivate 5 $\log_{10}$ cycles the population of *Salmonella* (Figure 3b). Thus, for example, 59.3 °C/3.5 min following 0.5 kGy, 58.4 °C/3.5 min following 1.0 kGy, or 57.4 °C/3.5 min following 1.5 kGy would enable achievement of a 5-$\log_{10}$ *Salmonella* reduction in LWE. In any case, this reduction of the process criterion only could be considered unless the reparation of the cell injury is prevented, by, for example, using additional hurdles after treatments (that is, cooling, antimicrobials, and so on).

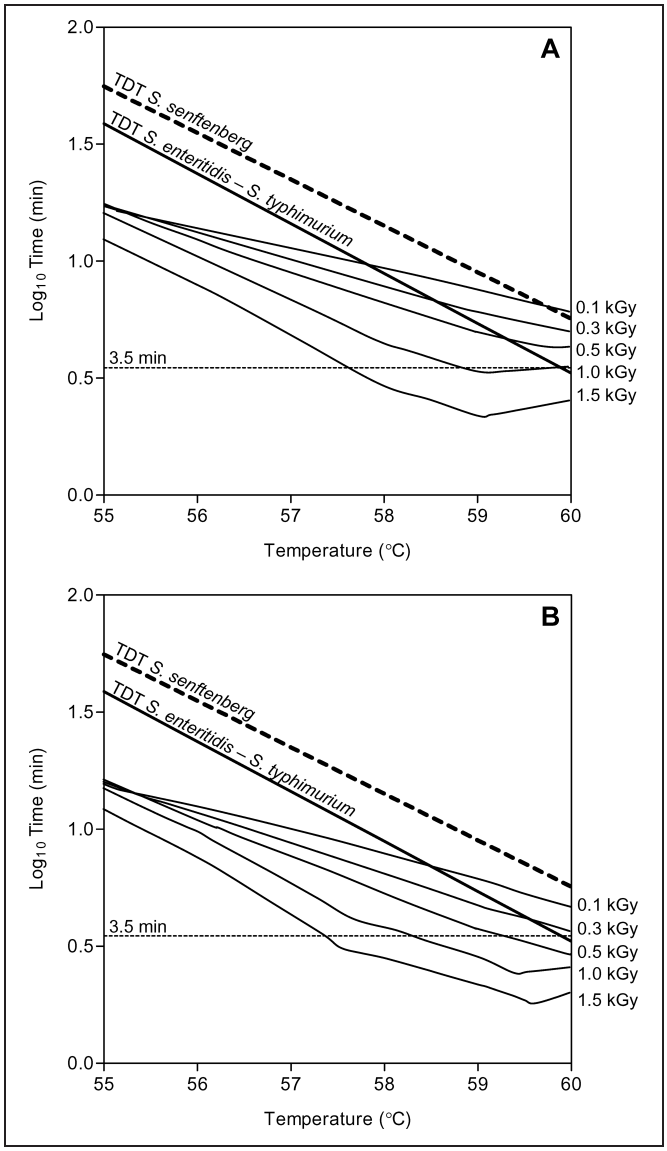

Figure 3—Process criteria to reduce 5-$\log_{10}$ the population of *Salmonella* by IR-H treatments in LWE (thin solid lines) and recovered in (A) nonselective and (B) selective media. 5-$\log_{10}$ reduction TDT curves for *Salmonella* Enteritidis, *Salmonella* Senftenberg, and *Salmonella* Typhimurium have been included. A 3.5-min-treatment time line has been plotted to compare treatments (thin dotted line).

Conclusions

As it can be observed, IR-H treatments would reduce the heat treatment intensities that may reduce the impact of the heat treatment on the LWE freshness quality. However, it is necessary to carry on investigations related to the influence of these IR-H treatments on the quality and functional properties of LWE. On the other hand, the existence of cell injury after IR-H treatments could suppose a larger microbial inactivation during storage of pasteurized LWE at refrigerated temperatures since refrigeration temperature is one of the most effective means to prevent the repair of irradiation- and heat-injured cells (Borsa and others 1995; Chawla and others 1996). IR-H-induced cell injury also opens new possibilities of combining processes using additives with antimicrobial activity (that is, nisin, organic acids, and so on), which could increase the lethal effectiveness of the IR-H process in LWE.

The models developed in this study can be applied to engineering design, and for the evaluation and optimization of the IR-H process as a new technique to obtain *Salmonella*-free LWE. Further studies are necessary to determine if the process and the models developed are also valid for other *Salmonella*, to evaluate the impact of the process on the quality and functional properties of LWE, and to determine the possibility of extending the use of the hurdle technology in the LWE industry based on IR-H-induced cell injury

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